Orphan G protein-coupled receptors: a neglected opportunity for pioneer drug discovery

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Access to DNA databases has introduced an exciting new dimension to the way biomedical research is conducted. 'Genomic research' offers tremendous opportunity for accelerating the identification of the cause of disease at the molecular level and thereby foster the discovery of more selective medicines to improve human health and longevity. The current challenge is to close the gap rapidly between gene identification and clinical development of efficacious therapeutics. In the present review, **Jeffrey Stadel, Shelagh Wilson and Derk Bergsma** outline the rationale and describe strategies for converting one large class of novel genes, orphan G protein-coupled receptors (GPCRs), into therapeutic targets. Historically, the superfamily of GPCRs has proven to be among the most successful drug targets and consequently these newly isolated orphan receptors have great potential for pioneer drug discovery.

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The advent of rapid DNA sequencing spawned the 'genomic era', which has led to the initiation of the Human Genome Project. The novel technologies developed in association with genomic research have already had a significant impact on the way investigations into the basis of disease are being conducted and will, no doubt, substantially enhance the means by which diseases are diagnosed and treated in the near future. To keep pace with the evolution of molecular medicine, the pharmaceutical industry has embraced genomics and is attempting to exploit the new technologies to identify novel targets for drug discovery. The major questions that remain to be addressed concern how to convert genomic sequences into therapeutic targets in an expeditious manner and eventually to obtain pharmaceutical drugs that will enhance the quality of life. This review will deal with a single class of novel molecular targets, focusing on the burgeoning collection of G protein-coupled receptors (GPCRs) called 'orphan' receptors[1]. GPCRs are a superfamily of integral plasma membrane proteins involved in a broad array of signalling pathways. Since the first cloning of GPCR gene sequences over a decade ago, novel members of the GPCR superfamily have continued to emerge through cloning activities as well as through bioinformatic analyses of sequence databases, although their ligands are unidentified and their physiological relevance remain to be defined. These 'orphan' receptors provide a rich source of potential targets for drug discovery.

The members of the GPCR superfamily are related both structurally and functionally. The signature motif of these receptors is seven distinct hydrophobic domains, each of which is 20–30 amino acids long and which are linked by hydrophilic amino acid sequences of varied length[2,3]. Biophysical[4] and biochemical[5] studies support the notion that these receptors are intercalated into the plasma membrane with the amino terminus extracellular and the carboxy terminus in the cytoplasmic portion of the cell. Therefore, these receptors are often referred to as seven transmembrane (or 7TM) receptors. While it is not yet known how many individual genes actually encode these receptors, it is clear that this family of proteins is one of the largest yet identified. Functionally, GPCRs share in common the property that upon agonist binding they transmit signals across the plasma membrane through an interaction with heterotrimeric G proteins[6,7]. These receptors respond to a vast range of agents[2,5,8] such as protein hormones, chemokines, peptides, small biogenic amines, lipid-derived messengers, divalent cations (e.g. a Ca^{2+} sensor has been identified that is a GPCR)[9] and even proteases such as thrombin, which activates its receptor by cleaving off a portion of the amino terminus[10]. Finally, these receptors play an important role in sensory perception including vision and smell[2,5,8]. Correlated with the broad range of agents that activate these receptors is their existence in a wide variety of cells and tissue types, indicating that they play roles in a diverse range of physiological processes. It is likely, therefore, that the GPCR superfamily is involved in a variety of pathologies. This point was recently emphasized by the surprising discovery that certain GPCRs for chemokines act as co-factors for HIV infection[11–13].

GPCRs represent the primary mechanism by which cells sense alterations in their external environment and convey that information to the cells' interior. The binding of an agonist to the receptor promotes conformational changes in the cytoplasmic domains that lead to the interaction of the receptor with its cognate G protein(s). Agonist-promoted coupling between receptors and G proteins leads to the activation of intracellular effectors that substantially amplify the production of second messengers feeding into the signalling cascade. Since effectors are often enzymes [e.g. adenylate cyclase[14], which converts ATP to cAMP, or phospholipase C (Ref. 15), which hydrolyses inositol lipids in membranes to release inositol trisphosphate, which in turn mobilizes Ca^{2+} within a cell] or ion channels[16], many second messenger molecules can be produced as the result of a single agonist binding event with its receptor. Changes in the intracellular levels of ions or cAMP, or both,

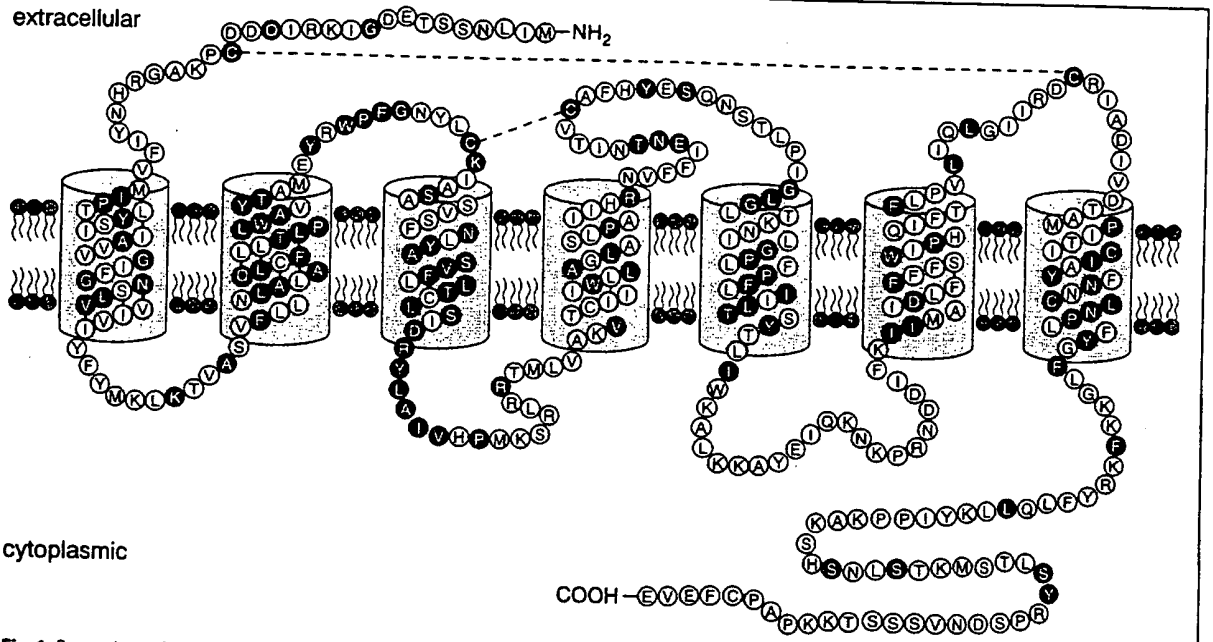

Fig. 1. Comparison of the protein sequence identity of the orphan APJ[19] receptor with the angiotensin AT_1 receptor[20]. The filled circles indicate amino acid identity (29.9%) between the two G protein-coupled receptors (GPCRs). This is a typical example of the protein sequence identity shared between orphan and known GPCRs.

result in the modulation of distinct phosphorylation cascades[17,18], extending through the cytosol to the nucleus, that eventually culminate in the physiological response of the cell to the extracellular stimulus. Although the overall paradigm is apparently the same for all GPCRs, the diversity of receptors, G proteins and effectors suggest a myriad of potential signalling processes and this becomes an important concept as we try to identify the function of orphan GPCRs.

To date, more than 800 GPCRs have actually been cloned from a variety of eukaryotic species, from fungi to humans [see L. F. Kolakowski in GCRDb-WWW The G Protein-Coupled Receptor DataBase World-Wide-Web Site (http://receptor.mgh.harvard.edu/GCRDBHOME.html.org)]. For humans, the most represented species, about 140 GPCRs have been cloned for which the cognate ligands are also known. This number excludes the sensory olfactory receptors, of which hundreds to thousands are predicted to exist. By traditional molecular genetic approaches, coupled with the explosion in genomic information, it has been possible to identify more than 100 additional orphan GPCR family members. By definition, there is enough sequence information in the receptor cDNAs to place them clearly in the superfamily of GPCRs, but often there is insufficient sequence homology with known members of this family to be able to assign their ligands with confidence or predict their function. In total, there are currently over 240 human GPCRs, excluding sensory receptors. As the size of sequence databases continues to increase, this list is expected to grow to 400, and perhaps even to 1000 or more unique gene products. The list will grow even further as paralogues and alternatively spliced GPCR variants emerge. Most orphan GPCRs share a low degree of sequence homology (typically about 25–35% overall amino acid sequence identity), with known GPCRs, suggesting that they belong to new subgroups of receptors (Fig. 1)[19,20]. Indeed, several orphan GPCRs show closer homology to each other than to known GPCRs. Nevertheless, the majority of orphan receptors are phylogenetically distributed among a broad spectrum of distantly related, known receptor subgroups.

What is the rationale for investing considerable time and resources into trying to establish the function of orphan GPCRs? Simply stated, GPCRs have a proven history of being excellent therapeutic targets. Within the past 20 years, several hundred new drugs have been registered that are directed towards activating or antagonizing GPCRs; in fact, it is estimated that most current research within the pharmaceutical industry is focused on this signalling pathway[21]. Table 1 shows a representative snapshot of a variety of receptors, disease targets and corresponding drugs. It is clear from this table that the therapeutic targets span a wide range of disorders and disease states. Another example of the significance and versatility of GPCRs is the number of cases of genetic diseases that are linked to defects in these proteins; some of these diseases are indicated in Table 2 (Refs 22–38). It is likely that many more genetic diseases will be mapped to GPCRs as the era of genomics continues to expand and families with inherited mutations are examined much more comprehensively.

The importance of GPCRs to drug discovery continues to be manifested by the fact that across the pharmaceutical industry active research projects, ranging from basic studies all the way through to advanced development, are focused on GPCRs as primary targets. Molecular biology has had a dramatic influence on these efforts.

Table 1. Examples of marketed drugs for G protein-c upled receptors (GPCRs)

GPCR	Generic	Drug	Indication
Muscarinic acetylcholine	Bethanechol	Urecholine	GI
	Dicyclomine	Bentyl	GI
	Ipratropium	Atrovent	CP
Adrenoceptor			
β_1	Atenolol	Tenormin	CP
α_2	Clonidine	Catapres	CP
β_1/β_2	Propranolol	Inderal	CP
α_1	Terazosin	Hytrin	CP
β_2	Albuterol	Ventolin	CP
$\beta_1/\beta_2/\alpha_1$	Carvedilol	Coreg	CP
Angiotensin			
AT_1	Losartan	Cozaar	CP
	Eprosartan	Teveten	CP
Calcitonin	Calcitonin	Calcimar	Osteoporosis
	eel-Calcitonin	Elcatonin	Osteoporosis
Dopamine			
D_2	Metoclopramide	Reglan	GI
D_2/D_3	Ropinirole	Requip	CNS
D_2	Haloperidol	Haldol	CNS
Gonadatropin-releasing factor	Goserelin	Zoladex	Cancer
	Nafarelin	Synarel	Endometriosis
Histamine			
H_1	Dimenhydrinate	Dramamine	CNS
H_1	Terfenadine	Seldane	CP
H_2	Cimetidine	Tagamet	GI
H_2	Ranitidine	Zantac	GI
Serotonin (5-HT)			
$5\text{-}HT_{1D}$	Sumatriptan	Imitrex	CNS
$5\text{-}HT_{2A}$	Ritanserin	Tisertan	CNS
$5\text{-}HT_4$	Cisapride	Propulsid	GI
$5\text{-}HT_{1B}$	Trazodone	Desyrel	CNS
$5\text{-}HT_{2A/2C}$	Clozapine	Clozaril	CNS
Leukotriene	Pranlukast	Onon	CP
	Zafirlukast	Accolate	CP
Opioid			
κ	Buprenorphine	Buprenex	CNS
	Butorphanol	Stadol	CNS
μ	Alfentanil	Alfenta	CNS
	Morphine	Kadian	CNS
Oxytocin		Syntocinon	Labour
Prostaglandin	Epoprostenol	Flolan	CP
	Misoprostol	Cytotec	GI
Somatostatin	Octreotide	Sandostatin	Cancer
Vasopressin	Desmopressin		CP/Renal

CP, cardiopulmonary system; GI, gastrointestinal system.

Table 2. Diseases associated with mutations of G pr tein-coupled receptors (GPCRs)

GPCR	Mutation	Disease	Refs
Rhodopsin	Missense: Pro23 to His (NT) Missense: Val87 to Asp (2TM) Missense: Tyr178 to Cys (2EL) Nonsense: Gln344 to Stop (CT)	Retinitis pigmentosa	22, 23
Thyroid stimulating hormone	Missense: Asp619 to Gly (3IL) Missense: Ala623 to Ile (3IL)	Hyperfunctioning thyroid adenomas	24
Luteinizing hormone	Missense: Asp578 to Gly (6TM)	Precocious puberty	25
Vasopressin V_2	Missense: Arg137 to His (2IL) Missense: Gly185 to Cyc (2EL) Frameshift at Arg230 (3TM)	X-linked nephrogenic diabetes	26–28
Ca^{2+}	Missense: Arg186 to Glu (NT) Missense: Glu298 to Lys (NT) Missense: Arg796 to Trp (3IL) Missense: Glu128 to Ala (NT)	Hyperparathyroidism, hypocalciuric hypercalcaemia	29, 30
Parathyroid hormone (PTH type b)	Missense: His223 to Arg (1IL)	Short-limbed dwarfism	31
β_3-Adrenoceptor	Missense: Trp64 to Arg (1IL)	Obesity, NIDDM	32–34
Growth-hormone-releasing hormone	Nonsense: Glu72 to Stop (NT)	Dwarfism	35
Adrenocorticotropin	Missense: Ser74 to Ile (2TM)	Glucocorticoid deficiency	36
Glucagon	Missense: Gly40 to Ser (NT)	Diabetes, hypertension	37, 38

Abbreviations: CT, carboxyl terminus; EL, extracellular loop; IL, intracellular loop; NIDDM, non-insulin-dependent diabetes mellitus; NT, amino terminus; TM, transmembrane segment.

The cloning of cDNAs for well-known GPCRs led to the discovery of a surprising number of paralogues[5]. The existence of these novel receptor subtypes was unexpected because the current cornucopia of pharmacological agents does not possess the required selectivity to distinguish all of them clearly, and thus an opportunity for drug discovery was quickly recognized. Current research efforts seek to define the physiology associated with these novel receptor subtypes and to discover highly selective compounds as potential pharmaceutical drugs. These efforts are almost exclusively focused on GPCRs for which activating ligands are known. Since characterized GPCRs were, and continue to be, attractive therapeutic targets, it is most reasonable to speculate that many of the orphan receptors have similar potential. The initial challenge is to determine the function of each orphan receptor through the identification of activating ligands and, once the function is clarified, link the orphan receptor to a specific disease and thus establish it as a candidate for a comprehensive drug discovery effort.

Reverse molecular pharmacology

Until recently, research into the identification of GPCRs as targets for drug discovery has been conducted using the traditional approach illustrated in Fig. 2. For this strategy, the starting point is functional activity, which forms the basis of an assay by which a ligand is identified through purification from biological fluids, cell supernatants or tissue extracts. One example of the success of this strategy is the discovery of the potent vasoconstricting peptide endothelin[39]. Once isolated, the ligand is used to characterize its cellular and tissue biology as well as its pathophysiological role. Subsequently, cDNAs encoding corresponding receptors are 'fished' from gene libraries using a variety of methodologies (e.g. receptor purification and expression cloning) that often either directly or indirectly use the ligand as the 'hook'. As the nucleotide sequences for GPCRs begin to accumulate and be analysed, additional receptors can be cloned by homology screening, by positional cloning, and by polymerase chain reaction (PCR) methodologies that use oligonucleotide primers based on nucleotide sequences conserved within the seven transmembrane domains of the GPCR family. Once the cloned human receptor cDNA is expressed in a heterologous cell system[40], it is used, together with its ligand, to form the basis of a screen to explore chemical compound libraries for receptor antagonists or agonists. Lead structures identified in the screen are refined through medicinal chemistry using an iterative process. Resulting drug leads with appropriate *in vivo* pharmacology are passed on into the clinic for development.

Recently, this paradigm has changed radically with the introduction of a new reverse molecular pharmacological

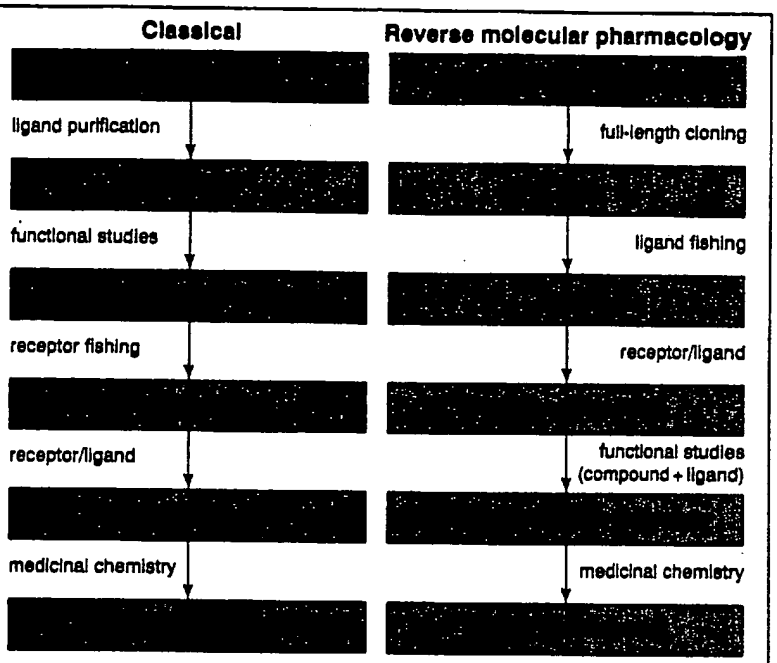

Fig. 2. Paradigm shift from classical to reverse molecular pharmacological approaches for drug discovery.

strategy, shown diagramatically in Fig. 2. Through both traditional molecular cloning techniques and, more recently, mass sequencing of expressed sequence tags (ESTs) from cDNA libraries, it is now possible to identify GPCRs through computational or bioinformatic methodologies. The EST approach, initially proposed by Sidney Brenner (University of Cambridge) and first brought to large-scale practice by Craig Venter (The Institute of Genome Research), constitutes random, single-pass sequencing of cDNAs randomly picked from a collection of cDNA libraries, followed by extensive bioinformatic analysis of the sequence to identify structural signatures characteristic of GPCRs. Once new members of the GPCR superfamily are identified, the recombinantly expressed receptors are used in functional assays to search for the associated novel ligands. The receptor–ligand pair are then used for compound bank screening to identify a lead compound that, together with the activating ligand, is used for biological and pathophysiological studies to determine the function and potential therapeutic value of a receptor antagonist (or agonist) in ameliorating a disease process. In addition, clues as to therapeutic potential may involve receptor genotyping of disease populations. Once a link with a disease is finally identified, an appropriate compound can be advanced for clinical study.

The reverse molecular pharmacological strategy is a far more daunting challenge and risky endeavour when compared with the more traditional approach, since the starting material for a drug discovery effort is simply an orphan receptor of unknown function, with no apparent relationship to a disease indication. However, the potential reward of using this approach is that resultant drugs naturally will be pioneer or innovative discoveries, and a significant proportion of these unique drugs may be useful to treat diseases for which existing therapies are lacking or insufficient.

Screening strategy

Figure 3 illustrates the generic strategy that we use for our reverse molecular pharmacological approach. In addition to the EST approach, which has yielded the majority of our collection of orphan receptors, we have also used a number of more traditional approaches such as low-stringency screening, using portions of known GPCRs as hybridization probes, as well as PCR-based methods. By these techniques we have succeeded in identifying more than 70 orphan receptors in addition to those already cited in the literature.

Since cDNAs identified by EST cloning are often incomplete, northern hybridization analysis is used to establish the tissue or cell pattern of mRNA expression of the GPCRs. This information is used to identify the tissue or cell cDNA libraries that are to be probed for full- length clones and, significantly, to determine whether a receptor is expressed in a particular normal or diseased tissue of interest. A highly selective tissue expression pattern may also provide a clue with respect to receptor function. Once obtained, full-length GPCR clones are expressed in mammalian cell lines and yeast model systems (see below) for functional analysis. *Xenopus* oocytes may also be used for expression; however, low screening throughput limits their use to a secondary, confirmatory assay system. For mammalian cell expression, the human embryonic kidney (HEK) 293 cell line or Chinese hamster ovary (CHO) cells are frequently used. These cell types possess a large repertoire of G proteins that are necessary for coupling to downstream effectors *in situ*. They also share a reliable history of positive functional coupling for a wide variety of known GPCRs. However, since receptor coupling cannot be accurately predicted from primary sequence data, orphan GPCRs may need to be expressed in a variety of cell lines to establish viable coupling.

These heterologous expression systems form the basis for screening for an activating ligand. The success of establishing functional coupling of the recombinant receptor depends to a large extent on whether the receptor is properly expressed, which may be assessed by northern or Western blot analysis, and whether appropriate G proteins and downstream effectors are present in the cell in which the receptor is expressed. There are several major technical challenges to be met in order to initiate ligand fishing. Because it is difficult to predict accurately the coupling specificity of orphan GPCRs from their primary sequence, assays must be chosen that will detect a wide range of coupling mechanisms. These generally focus on changes in intracellular levels of cAMP or Ca^{2+} but can also include more generic measurements, such as metabolic activation of the cell via the cytosensor microphysiometer[41]. Recently, it has become possible to implement most of these screens in high-throughput format by using fluorescent-based

assays and using charge-coupled device cameras and reporter gene constructs that allow easy readout of the assay on microtitre plates. Ever increasing throughput of the assays will be necessary to screen large libraries. However, this approach is somewhat cumbersome and inefficient if all the assays described above have to be used. Is it possible to funnel heterologous signal transduction through a defined pathway? The prospect of an assay for a single transduction pathway comes from the observation that heterologous expression of the G protein subunit $G_{\alpha 15/16}$ promoted coupling of various GPCR subfamily members through activation of phospholipase Cβ and likely Ca^{2+} mobilization[42,43]. Although this approach may not work universally, the diversity of the GPCRs successfully coupled through $G_{\alpha 16}$ to phospholipid metabolism suggests that this could be a useful method to screen for orphan receptor activation.

Once heterologous receptor expression is achieved and functional assays are in place, ligand fishing experiments can be initiated. Although the homology with known GPCRs is low, we nevertheless begin by screening the orphans against known GPCR ligands; since the sequence homology between some subtypes of known receptors can be low (e.g. 30–40% between neuropeptide Y receptor subtypes), it is possible that new paralogue receptors for known ligands still remain to be discovered. The next step is to search for novel activating ligands by screening biological extracts obtained from tissues, biological fluids and cell supernatants. An additional option is screening libraries of compounds for activating ligands. Complex libraries of peptides or compound collections could be rich sources of 'surrogate' agonists that would promote receptor activation and coupling but are not endogenous ligands. The rationale for searching for surrogate agonists springs from a report that a nonpeptide agonist has been discovered for the angiotensin II receptor[44]. There is also an obvious precedent for nonpeptide agonists for opioid receptors. Screening of the very large libraries that will be generated by fractionation of biological extracts and by combinatorial chemical synthesis requires that the functional assays used have not only a high throughput but are also robust, since false positives can be a significant problem.

Examples are beginning to emerge from several efforts showing that progress has been made in characterizing orphan GPCRs. A first example is the identification of an orphan GPCR that functions as a calcitonin gene-related peptide (CGRP) receptor[45]. CGRP is a peptide of 37 amino acids, widely distributed in neurones, and functions as a potent vasodilator. It may be involved in migraine and has been implicated in non-insulin-dependent diabetes mellitus because it promotes resistance to insulin. An orphan GPCR EST was derived from a human synovium cDNA library[45]. Sequence analysis showed that the new GPCR has ~56% similarity to the human calcitonin receptor and was hence originally expected to be a new subtype of the calcitonin receptor. The message for this novel receptor was expressed predominantly in lung, which is known to be a relatively rich source of CGRP receptors. Following full-length cloning from a human lung library, the orphan receptor cDNA was stably expressed in HEK293 cells. Both radioligand binding using ^{125}[I]CGRP, as well as functional assays of CGRP-stimulated cAMP accumulation, demonstrated an appropriate pharmacological profile for the expressed receptor similar to that observed with endogenous CGRP receptors on human neuroblastoma cells. In addition to identifying the CGRP receptor, the reverse molecular pharmacology approach has also been used to identify other orphan receptors, such as the anaphylatoxin C3a receptor[46].

The examples given above are for receptors with significant homology to known GPCR superfamily members and their activating ligands proved to be known GPCR ligands. Will ligand fishing be successful in identifying novel endogenous ligands? Recently, two groups

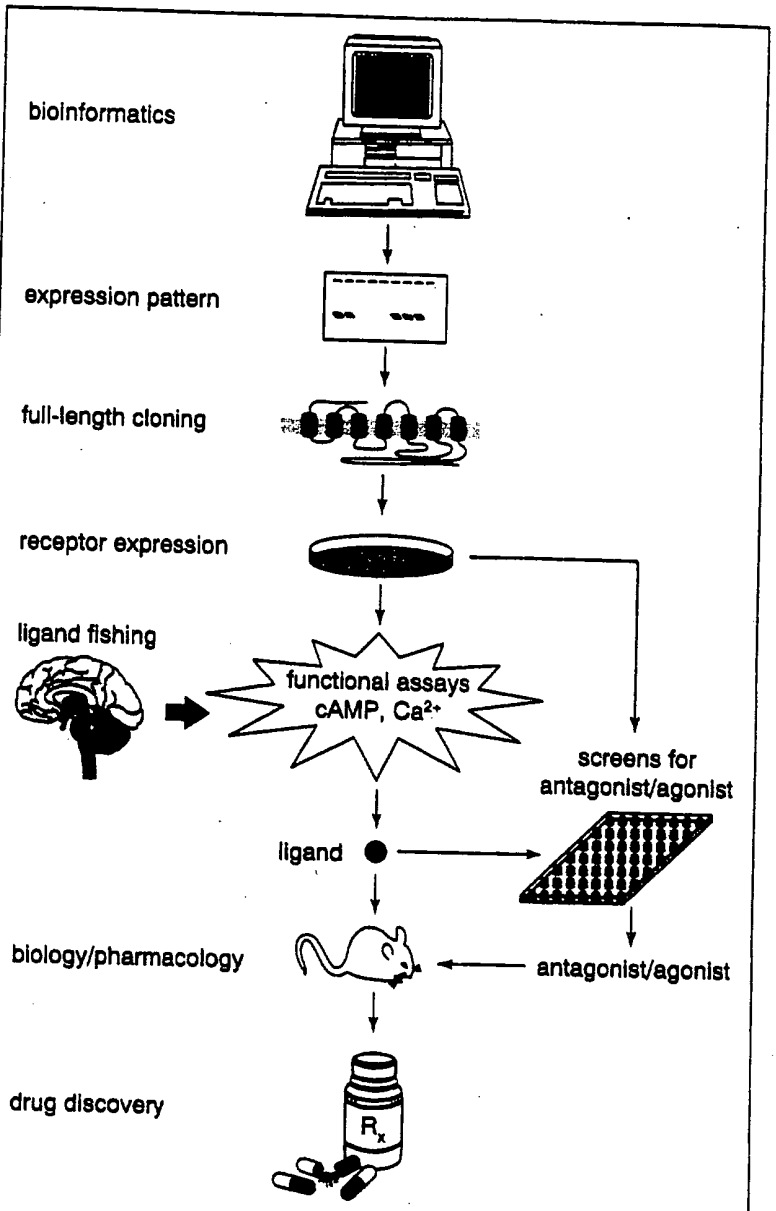

Fig. 3. Strategy for using orphan G protein-coupled receptors as targets for drug discovery.

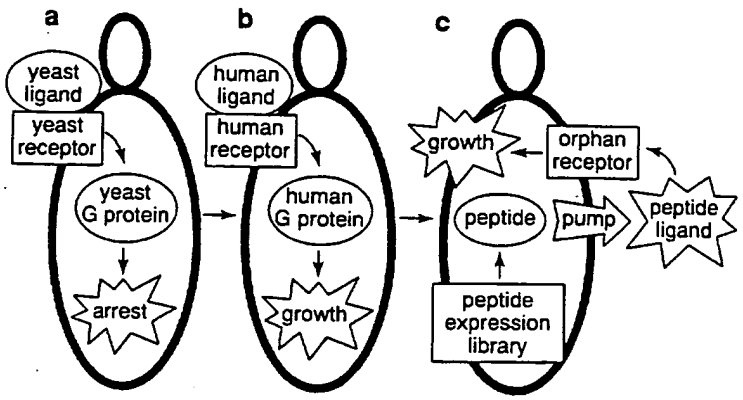

Fig. 4. Yeast-based screen for the identification of agonists for orphan G protein-coupled receptors (GPCRs). **a:** Normal, endogenous GPCR signalling in yeast (*Saccharomyces cerevisiae*). **b:** Substitution of a human GPCR and a human G_α subunit for yeast counterparts and modification of downstream signalling pathways such that agonist stimulation of the recombinant GPCR promotes growth. This yeast strain can be screened using biological extracts or compound libraries, or both. **c:** Yeast cells can be engineered to secrete small peptides from a random peptide library to identify autocrine surrogate, peptide agonists for recombinant orphan GPCRs. Modified from Ref. 49, with permission.

investigated an orphan opioid-like receptor, ORL1 (Refs 47 and 48). Both groups expressed the orphan GPCR in CHO cells and challenged the transfected cells with a series of opiate agonists, but without response. Both groups then used a similar ligand fishing approach. Taking crude extracts from rat brain[47] or porcine brain[48], they screened against the stably transfected cell lines using inhibition of adenylate cyclase activity as a functional assay. They were able to fractionate the brain extracts and identify the novel dynorphin-like ligand, which they called nociceptin[47] or orphanin FQ (Ref. 48). Thus, both teams successfully established a functional assay in transfected CHO cells that allowed the purification of a novel neuropeptide ligand that is 17 amino acids long for the orphan receptor. This work validates the ligand fishing approach for characterizing the function of orphan GPCRs.

Concluding remarks and future challenges

Although orphan GPCRs have been around for over ten years, very few companies have, until recently, been willing to risk their resources to explore opportunities among this category of receptors. However, the environment for the pharmaceutical industry has changed due to the confluence of several major technological advances. The conversion of gene sequences encoding GPCRs to drug targets is substantially aided by the development of combinatorial chemistry methods and miniaturized high-throughput screening techniques. The future challenge for drug discovery in this arena is to integrate these technologies innovatively and productively. One glimpse of the future comes from the field of functional genomics. The endogenous GPCR transduction system of the yeast, *Saccharomyces cerevisiae*, which is the pheromone pathway required for conjugation and mating, has been commandeered – through genetic engineering – to permit functional expression and coupling of human GPCRs and humanized G protein subunits to the endogenous signalling machinery[49–51] (Fig. 4). Further manipulations involve conversion of the normal yeast response to pheromone or activating ligand (growth arrest) to positive growth on selective media or to reporter gene expression. In addition, yeast cells have been engineered to express and secrete small peptides from a random peptide library that will permit the autocrine activation of heterologously expressed human GPCRs (Refs 49 and 51). This provides an elegant means of screening rapidly for surrogate peptide agonists that activate orphan receptors. This yeast system is, of course, not limited to autocrine ligand screening but can also be used in high-throughput mode to screen directly the fractions from biological extracts and the various chemical libraries as described above. A major advantage of the yeast system over the mammalian heterologous expression systems is its ease of use and its lack of endogenous GPCRs, which can confound ligand fishing expeditions in mammalian cells.

There is now tremendous pressure to be the first on the market with highly selective drugs that target therapeutic areas of unmet medical need and ideally have novel mechanisms of action. As a consequence, the pharmaceutical industry has recognized the power of genomics to provide it with new and unique drug targets. Genomics has responded with a plethora of novel proteins, included among them over 100 orphan GPCRs. Because of the proven link of GPCRs to a wide variety of diseases and the historical success of drugs that target GPCRs, we believe that these orphan receptors are among the best targets of the genomic era to advance into the drug discovery process.

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CA_1A_2X-competitive inhibitors of farnesyltransferase as anti-cancer agents

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For Ras oncoproteins to transform mammalian cells, they must be post-translationally farnesylated in a reaction catalysed by the enzyme farnesyl-protein transferase (FPTase). Inhibitors of FPTase have therefore been proposed as anti-cancer agents. In this review **Charles Omer and Nancy Kohl** discuss the development of FPTase inhibitors that are kinetically competitive with the protein substrate in the farnesylation reaction. These compounds are potent and selective inhibitors of the enzyme that block the tumourigenic phenotypes of *ras*-transformed cells and human tumour cells in cell culture and in animal models.

Since the identification of farnesyl-protein transferase (FPTase) activity in mammalian cells, there has been an intense effort to develop inhibitors of this housekeeping enzyme for use as potential, novel anti-cancer agents[1,2]. This idea stems from the fact that several of the proteins that regulate mammalian cell proliferation require a post-translational modification catalysed by this enzyme for biological activity. Efforts over the past eight years have yielded potent, cell-active inhibitors of FPTase that demonstrate anti-proliferative activity in cell culture and in rodent models of cancer.

The focus of the FPTase inhibitor (FTI) studies has been inhibition of the transforming activity of the Ras oncoproteins. Three *ras* genes, Ha-, N- and Ki-*ras*, encode four highly homologous, 21 kD proteins, Ha-, N-, Ki4A- and Ki4B-Ras (Ki4A- and Ki4B-Ras are encoded by splice variants of the Ki-*ras* gene)[3]. Ras functions to regulate the transduction of extracellular growth-promoting signals from membrane-bound receptor tyrosine kinases to intracellular growth-regulatory pathways. Typical of the low-molecular-weight G proteins, Ras is active when bound to GTP and inactive when bound to GDP. Cycling from the active to the inactive form is accomplished by the intrinsic GTPase activity of the protein. Mutations in Ras that abolish the GTPase activity result in constitutively active forms of the protein. Such oncogenically mutated forms of Ras, particularly Ki4B-Ras, are found in approximately 30% of many human cancers including 90% of pancreatic cancers and 50% of colon cancers[4,5].

Ras is synthesized as a biologically inactive, cytosolic protein that localizes to the inner surface of the plasma membrane where it acquires biological activity following a series of post-translational modifications (see Ref. 6 for review). The first and obligatory step in this series is the transfer of a 15-carbon isoprenoid, farnesyl, from farnesyl diphosphate (FPP) to the sulphur atom of the cysteine residue located four amino acids from the carboxyl terminus of the protein. This cysteine residue is part of the CA_1A_2X motif found in all FPTase protein substrates, where C is cysteine, A_1 and A_2 are usually aliphatic amino acids and X is usually serine, methionine, glutamine, alanine or cysteine. Following farnesylation, A_1A_2X is proteolytically cleaved and the now C-terminal farnesylcysteine is methylated. In the case of all of the Ras proteins except Ki4B-Ras, palmitate groups are then added to cysteine residues upstream of the farnesylated cysteine. The demonstration that farnesylation is essential for the transforming ability of the Ras oncoproteins[7–10] has spurred the development of inhibitors of the enzyme that catalyses this reaction, FPTase, as anti-cancer agents.

FPTase is a ubiquitously expressed, cytosolic enzyme comprised of two subunits, a 45 kDa α subunit and a 48 kDa β subunit[6]. Cross-linking studies have shown

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